British Journal of Nutrition (2004), **91**, 749–755
DOI: 10.1079/BJN20041101
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Effects of esculin and esculetin on the survival of *Escherichia coli* O157 in human faecal slurries, continuous-flow simulations of the rumen and colon and in calves

Sylvia H. Duncan[1]*, E. Carol McWilliam Leitch[1], Karen N. Stanley[1], Anthony J. Richardson[1], Richard A. Laven[2], Harry J. Flint[1] and Colin S. Stewart[1]

[1]*Gut Microbiology and Immunology Division, Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK*
[2]*Scottish Agricultural College, St Mary's Industrial Estate, Dumfries DG1 1DX, UK*

(Received 21 May 2003 – Revised 1 December 2003 – Accepted 16 January 2004)

The human pathogen *Escherichia coli* O157:H7 is thought to be spread by direct or indirect contact with infected animal or human faeces. The present study investigated the effects of the plant coumarin esculin and its aglycone esculetin on the survival of a strain of *E. coli* O157 under gut conditions. The addition of these compounds to human faecal slurries and *in vitro* continuous-flow fermenter models simulating conditions in the human colon and rumen caused marked decreases in the survival of an introduced strain of *E. coli* O157. When four calves were experimentally infected with *E. coli* O157 and fed esculin, the pathogen was detected in five of twenty-eight (18 %) of faecal samples examined post-inoculation, compared with thirteen of thirty-five (37 %) of faecal samples examined from five control calves not fed esculin. Coumarin compounds that occur naturally in dietary plants or when supplemented in the diet probably inhibit the survival of *E. coli* O157 in the gut.

Plant coumarins: *Escherichia coli* O157: Rumen: Colon

Escherichia coli O157:H7 has emerged as a serious foodborne pathogen in the UK and other developed countries over the past 20 years. Human infection can result from the ingestion of as few as 100 viable bacterial cells (Griffin & Tauxe, 1991) and commonly results in bloody diarrhoea and haemorrhagic colitis. Potentially fatal extra-intestinal complications may occur, particularly in the young and the elderly, resulting in haemolytic uraemic syndrome and thrombolytic thrombocytopenic purpurea (Griffin & Tauxe, 1991). The gut of herbivorous ruminant animals such as cattle and sheep is the main reservoir of *E. coli* O157 and transmission to man occurs principally through contamination of the food chain, or of the environment, by faeces from infected animals (Kudva *et al.* 1998). Except in neonates (Dean-Nystrom *et al.* 1998), carrier animals usually do not show outward evidence of disease. Faeco-oral transmission is also a significant factor in the spread of this pathogen in human populations. From studies in Wales, Parry & Salmon (1998) calculated a household transmission rate of about 7 %.

Antibiotic therapy of *E. coli* O157 infection is controversial since it has been associated with an increased risk of haemolytic–uraemic syndrome (Wong *et al.* 2000) and, in some instances, appears to increase the risk of secondary infection (Neill, 1998). Furthermore, the use of antibiotics as growth promoters in animal husbandry is accompanied by an unacceptable risk of enhancing the spread of resistance genes (Flint *et al.* 1987; Barbosa & Levy, 2000; Shoemaker *et al.* 2001). Alternative approaches for controlling the shedding of *E. coli* O157 in sheep and cattle include the use of probiotics (Zhao *et al.* 1998) and dietary manipulation, for example, decreasing the amount of cereal grains fed to animals (Diez-Gonzalez *et al.* 1998). Plant coumarins are known to inhibit the growth of *E. coli* O157 (Duncan *et al.* 1998). These plant metabolites are found in grassland herbage and may occur commonly in the free state and as glycosides (Murray *et al.* 1982). Duncan *et al.* (1998) showed that coumarin inhibited the growth of *E. coli* O157 in pure culture and in batch-culture incubations of rumen contents. Under the conditions used, the coumarins had relatively minor effects on the predominant anaerobic faecal microflora.

The fermentative compartments of the gut, including the rumen and colon of sheep and cattle and the human colon, are populated predominantly by anaerobes which produce SCFA, carbon dioxide, methane and water by fermentation (Hungate, 1966). The population density of anaerobic bacteria ranges from about 10^8 to 10^{11} viable cells/ml depending on the gut site, the nature of the diet and the time elapsed after the ingestion of food (Wolin, 1981; Wilson, 1997; Russell & Rychlik, 2001). *E. coli* and other facultative bacteria normally comprise between 0·01 and 1 % of

Abbreviations: APB, anaerobic phosphate buffer; cfu, colony-forming units; DMSO, dimethyl sulfoxide; LB, Luria Bertani.

* **Corresponding author:** Dr S. H. Duncan, fax + 44 1224 716687, email shd@rri.sari.ac.uk

the total bacterial population of the gut (Hungate, 1966; Wilson, 1997). *E. coli* O157 colonises the rumen and colon of naturally infected ruminants. In cattle at slaughter at abattoirs in England, the incidence of *E. coli* O157 and the numbers of commensal *E. coli* were higher in the colon than in the rumen (Laven *et al.* 2003). Nonetheless it is clear that, following ingestion, *E. coli* O157 must pass though the rumen and the other gut compartments before colonising the lower gastrointestinal tract. Control measures aimed at reducing the survival of *E. coli* O157 in the rumen and colon may be useful in ultimately reducing shedding rates amongst animals.

Here we report the effects of the plant coumarin esculin and its aglycone esculetin on the survival of *E. coli* O157 in human faecal slurries, fermenter models simulating conditions in the human colon and in the rumen, and the effect on faecal shedding following the experimental infection of calves.

Materials and methods

Escherichia coli *strains, growth conditions, inocula preparation and tracking*

E. coli serogroup O157 (NCTC 12900) used in the present study (12900) does not carry verocytotoxin genes or an inducible lambdoid prophage (James *et al.* 2001). Thus the laboratory and animals studies such as those described in the present paper can be performed at Health and Safety Executive category 2 instead of category 3 level of containment. Access to the latter facilities is limited in the UK, reducing the opportunity for relevant investigations. Cultures were routinely grown in Luria Bertani (LB) broths (Sambrook *et al.* 1982) (5 ml) in glass Hungate tubes (Bellco Glass Inc., Vineland, NJ, USA). *E. coli* O157 (12900) was grown for the inoculation of the colon-simulating fermenter in 100 ml volumes of LB medium at 37°C for 18 h in a shaking incubator (150 rpm). Cultures were centrifuged at 2500 ***g*** for 15 min and washed in sterile anaerobic phosphate buffer (APB; 50 mM) containing 0·5 % (w/v) cysteine HCl as a reducing agent and prepared under O_2-free CO_2. Pelleted cells were re-suspended in 10 ml APB and inoculated through a port into the fermenter vessel, then washed through with a further 10 ml sterile buffer.

For inoculation of the rumen fermenters, frozen stock solutions of *E. coli* O157 (12900) were inoculated anaerobically into 10 ml broths of M2GSC medium (Miyazaki *et al.* 1997) and incubated for 24 h in an orbital shaking incubator (100 rpm, 37 ± 1°C). A sample of 500 μl of the culture was used to inoculate the rumen fermenters. The culture was inoculated through a port into the fermenter vessel.

For the experiments with calves, a rifampicin-resistant mutant of *E. coli* O157 (12900) was created by sub-culturing the strain in increasing concentrations of rifampicin. Single colonies were tested for growth in broth medium containing either 75 or 100 μg rifampicin/ml. The *E. coli* O157 rifampicin-resistant mutant (12900^R) was maintained on LB agar plates containing 75 μg rifampicin/ml.

Selective counting of bacterial groups

Anaerobic, facultative anaerobic and faecal coliform bacteria were enumerated during *E. coli* O157 survival experiments. Decimal serial dilutions of samples were prepared in 9 ml anaerobic diluting fluid or 900 μl PBS. Total anaerobic bacteria were enumerated on roll tubes (Bryant, 1972) of M2GSC medium containing 2·0 % (w/v) agar. Total facultative counts were made on Nutrient agar (Oxoid, Basingstoke, Hants, UK). The total coliforms were enumerated on MacConkey agar. *E. coli* O157 cells were enumerated on sorbitol MacConkey agar supplemented with cefixime tellurite (CT-SMAC; Oxoid, Basingstoke, Hants, UK) as described by Chapman & Siddons (1996) or on Rainbow agar (Biolog Inc., Hayward, CA, USA). Roll tubes were incubated for 72 h and the plates for 48 h at 38°C.

Effect of esculin and esculetin on survival of Escherichia coli *O157 on incubation with human faeces*

A faecal slurry (10 % (w/v) final concentration) was prepared by suspending freshly voided faeces from a healthy vegetarian adult male (50 years old) in anaerobic 50 mM-APB. Hungate culture tubes containing 10 % faecal slurry (9·75 ml) were inoculated with 100 μl *E. coli* O157 (12900) that had been incubated for 18 h at 38°C and then incubated at 38°C under O_2-free CO_2. Sub-samples (100 μl) were removed for enumeration at intervals as recorded later (p. 751). Esculin and esculetin were prepared as 1 M stock solutions in 80 % (v/v) dimethyl sulfoxide (DMSO) and appropriate levels were added to give final concentrations of 5 mM. The appropriate levels of DMSO were added to the control tubes. The incubations were conducted as three replicates for each treatment and counts were the average of three replicates at 24 h intervals for a period of 72 h.

Effect of esculetin on survival of Escherichia coli *O157 in a simulation of human colonic fermentation*

A continuous-flow single-stage fermenter based on the system of Macfarlane *et al.* (1989) was used to model human colonic fermentation. The working volume was 900 ml, the temperature was maintained at 37°C and the pH was maintained between 6·5 and 6·8. Anaerobiosis was maintained by means of a stream of O_2-free CO_2 into the head of the medium vessel, and N_2 into the fermenter vessel as described by Scott *et al.* (1998). The medium used contained xylan, amylopectin, arabinogalactan, pectin, potato starch, peptone water (Unipath, Basingstoke, Hants, UK), and haemin, bile salts and minerals (Macfarlane *et al.* 1989) with (per litre) 3·0 g $NaHCO_3$, 0·5 g cysteine HCl and 0·5 ml antifoam A (Sigma, Poole, Dorset, UK). The medium turnover rate for the fermenter vessel was 1/d, corresponding to a dilution rate of 0·042/h. The vessel was inoculated with a faecal suspension of 10 g freshly voided faeces, from a healthy adult male, suspended in 50 mM-APB (40 ml). Esculetin in 80 % (v/v) DMSO was added to the test vessel and the feed flask to give a final concentration of 10 mM-esculetin and 1·6 % (v/v) DMSO.

DMSO only was added to the control vessel and feed flask to give the same final concentration.

Rumen fermenter simulations

Rumen-simulating fermenters were set up using an apparatus similar to that of Teather & Sauer (1988), but with a reduced working volume of 550 ml. Inoculum was prepared from the rumen contents from three cannulated sheep fed a general-purpose diet (McKain *et al.* 1992). The rumen contents were pooled, diluted 1:1 with artificial saliva (McDougall, 1948) and 550 ml was added to duplicate fermenters. The general-purpose diet (5 g), which had been ground to pass through a 1 mm dry mesh, was added to the fermenter vessel once daily for the first 3 d and thereafter twice daily. The turnover rate of the fermenter contents was approximately 1·5/d.

E. coli O157 (12900) was added to the fermenter contents to give $3{\cdot}5 \times 10^4$ colony-forming units (cfu)/ml. Esculin in 80 % (v/v) DMSO was prepared as for the human colonic fermenter and was added to the test vessel on the fourth day. Viable counts were performed as described earlier (p. 750) to determine the population of total anaerobes, total facultative anaerobes and total coliforms. *E. coli* O157 cells were enumerated using Rainbow agar. The fermenters were maintained for 7 d (4 d of testing) and the experiment was repeated using the rumen contents from the same sheep.

Effect of feeding esculin on faecal shedding of Escherichia coli *O157 in experimentally infected calves*

Friesian–Holstein male calves, 3–4 months of age, were randomly allocated to two treatment groups. The calves were housed indoors in individual pens on concrete floors bedded with wood shavings that were cleaned out daily. A proprietary calf mix was fed twice daily, with the addition of 50 g esculin sprinkled on the diet in the case of the esculin-treated group.

Before the experiment began, the palatability of esculin was confirmed by feeding a calf 50 g esculin powder in 20 kg feed. Animals to be included in the experiment were first confirmed negative for the faecal excretion of *E. coli* O157 and rifampicin-resistant *E. coli*. On days 1 and 2 all ten calves were administered 10 ml of overnight LB culture containing approximately 1×10^9 cfu of the rifampicin resistant *E. coli* O157 (strain 12900^R)/ml. Five of these animals received esculin in their normal feed on days 1 to 5 inclusively. One of the animals in the group receiving esculin was found to have an unrelated gut malfunction and, as a result, was withdrawn from the experiment. Fresh faecal samples were collected in the morning and transported to the laboratory in chilled containers within 24 h. *E. coli* O157 (12900^R) cells were enumerated on MacConkey agar containing 50 μg rifampicin/ml. Representative rifampicin-resistant colonies were sub-cultured onto MacConkey sorbitol agar and tested with *E. coli* O157 latex test kit (Oxoid, Basingstoke, Hants, UK) to confirm that they were *E. coli* O157 and not spontaneous *E. coli* rifampicin-resistant mutants.

Short-chain fatty acid analysis by gas chromatography

SCFA were analysed as described by Richardson *et al.* (1989), except that the derivatisation conditions were modified so that 800 μl diethyl ether extract was treated with 100 μl *N*-methyl-*N*-t-butyldimethylsilyltrifluoroacetimide.

Results

Batch incubations with human faeces in vitro

Anaerobic batch cultures of 10 % (w/v) human faecal slurry (final concentration), inoculated with *E. coli* O157 with or without the addition of esculin or esculetin, were assessed for the survival of total anaerobes, total coliforms and *E. coli* O157. The numbers of coliforms fell by approximately 10-fold in the controls but by more than 1 000-fold in the presence of the plant metabolites (Fig. 1 (a)). Most significantly, the numbers of *E. coli* O157 cells fell sharply in the presence of the plant metabolites. After 72 h incubation there was a decrease of over 1000-fold in the numbers of *E. coli* O157 cells compared with the control incubations and a decrease of more than 10 000-fold compared with the initial count (Fig. 1 (b)).

During 72 h incubation, the number of anaerobes fell by about 50 % in the controls, 75 % in the presence of

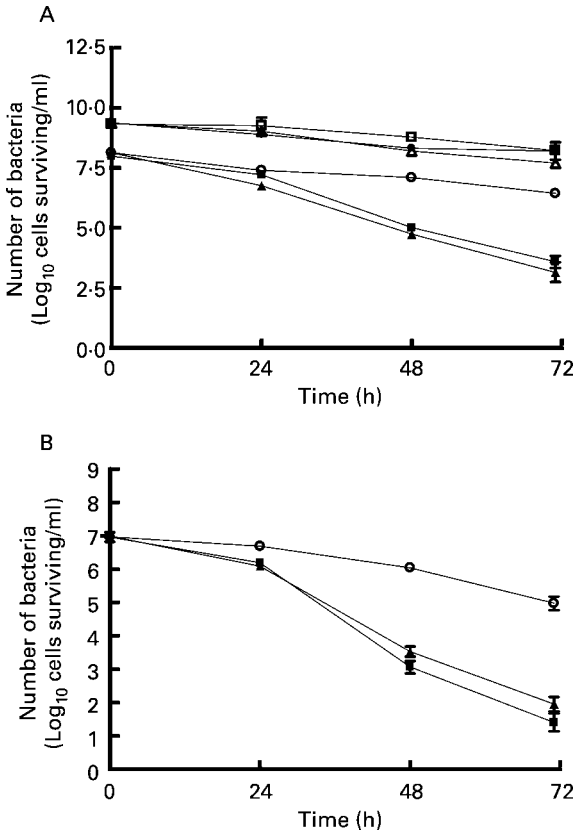

Fig. 1. (A) Effects of esculin and esculetin on the survival of anaerobic bacteria and total coliforms. (□), Anaerobes, control; (●), anaerobes with esculetin; (△), anaerobes with esculin; (○), coliforms, control; (■), coliforms with esuletin; (▲), coliforms with esculin. (B) *Escherichia coli* O157 in human faecal slurry in batch culture compared with controls with no added plant metabolites. (○), Control; (▲), esculetin; (■), esculin. Mean values are shown with their standard deviations represented by vertical bars.

esculetin, and 90 % in the presence of esculin (Fig. 1 (a)). The total SCFA concentrations at the end of the incubations for the control, esculetin- and esculin-containing batch cultures were (n 3): 43·0 (SD 5·1), 39·5 (SD 0·5) and 55·85 (SD 0·7) mM respectively. The final pH values of these incubations were 6·35 (SD 0·05) (control), 6·37 (SD 0·02) (esculetin) and 6·21 (SD 0·01) (esculin).

Human colon-simulating fermenters

A model system designed to simulate human colonic fermentation (37°C, pH 6·5, dilution rate 0·04/h) was used to study the effect of esculetin on the survival of *E. coli* O157 in the presence of human faecal bacteria in continuous culture.

In experiment 1, *E. coli* O157 was introduced on day 7 and esculetin (10 mM) was added on day 21 (Fig. 2). Between days 11 and 21, before the addition of esculetin, the average number of *E. coli* O157 present was 6·5 (SD 0·5) $\times 10^5$ cfu/ml (n 10). Following the addition of esculetin the numbers of *E. coli* O157 dropped to about 4·4 (SD 1·6) $\times 10^1$ cfu/ml (n 5) between days 26 and 31. Application of the t test showed that the population of *E. coli* in the control period (days 11 to 21) differed significantly from that during the esculetin-feeding period (26 to 31 d) ($P<0{\cdot}001$). When *E. coli* O157 was re-introduced in the presence of esculetin on day 31, its numbers fell sharply to below 10^2 cfu/ml by day 38 (Fig. 3). There was no evidence of an effect of esculetin on the numbers of anaerobic bacteria or of total coliforms (Fig. 2).

The results of a second experiment, in which test and control fermenters were run simultaneously with and without esculetin in the medium, are shown in Fig. 3. The reduction in the number of *E. coli* O157 in the fermenter treated with esculetin was significantly greater than that in the control vessel. Linear regression analysis (Genstat; Lawes Agricultural Trust, Harpenden, Herts, UK) was used to determine the survival of *E. coli* O157 in the control fermenter compared with the esculetin-fed fermenter.

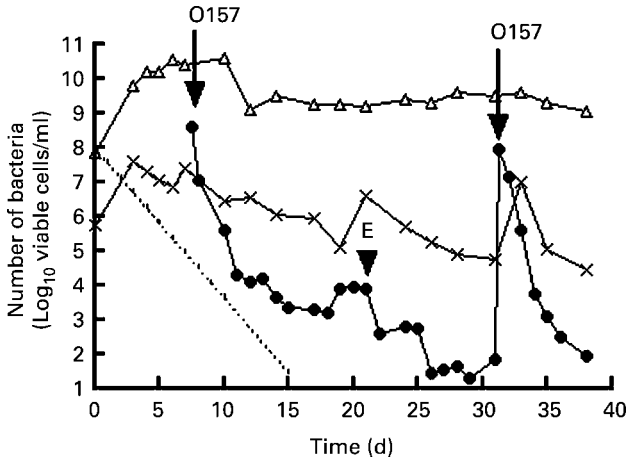

Fig. 2. Numbers of anaerobic bacteria (△), total coliforms (×) and *Escherichia coli* O157 (●) in a continuous-flow fermenter simulating human colonic fermentation. Mixed human faecal bacteria were introduced on day 0, and *E. coli* O157 on days 7 and 31. Esculetin (E; final concentration 10 mM) was added to the fermenter contents and the medium on day 21. (···), Washout rate.

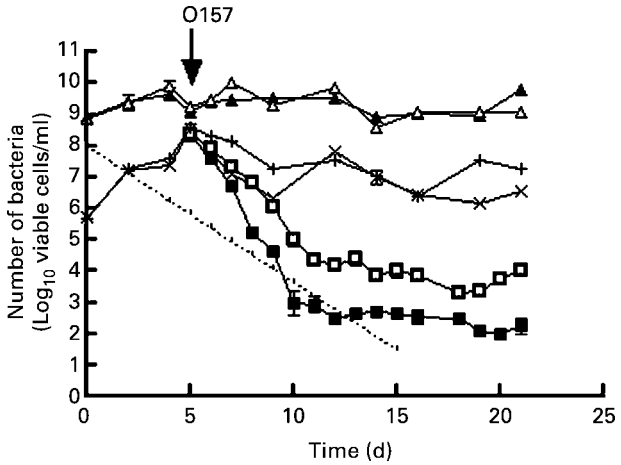

Fig. 3. Numbers of bacteria in a continuous-flow fermenter simulating human colonic fermentation. Mixed human faecal bacteria were introduced on day 0; *Escherichia coli* O157 on day 5. Fermenter 1 contained a control medium; fermenter 2 contained the medium plus 10 mM-esculetin. (△), Anaerobic bacteria in fermenter 1; (▲), anaerobic bacteria in fermenter 2; (+), total coliforms in fermenter 1; (×), total coliforms in fermenter 2; (□), *E. coli* O157 in fermenter 1; (■), *E. coli* O157 in fermenter 2; (···), washout rate.

ANOVA over time was used to compare the decline in numbers of *E. coli* O157 (12900) between the control and treatment vessels and this gave a value of $P=0{\cdot}004$. As in the previous experiment shown in Fig. 3, esculetin did not appear to affect the total numbers of anaerobes and coliforms. The mean concentration of SCFA in representative samples taken during the study was 56·9 (SD 4·6) mM (n 15); adding esculetin to the medium did not significantly change SCFA formation (average 59·2 (SD 5·4) mM; n 15). The molar proportions of the three major SCFA acetate, propionate and butyrate averaged about 4:2:1 throughout the study and were not significantly affected by the presence of esculetin (data not shown).

Rumen-simulating fermenter

E. coli O157 (12900) was added to duplicate rumen fermenter vessels and allowed to stabilise for 4 d before the addition of esculin to one vessel. The initial inoculum of *E. coli* O157 was 3·5 $\times$ 10^4 cfu/ml but the number increased in the first 4 d to an average of $1{\cdot}2 \times 10^6$ cfu/ml. This number was maintained for the remainder of the experiment in the control vessels but, in the test vessels, the number of *E. coli* O157 (12900) decreased after the addition of esculin to a minimum of $4{\cdot}8 \times 10^1$ cfu/ml by day 6 (Fig. 4).

In the test vessels, the numbers of anaerobic bacteria increased for the first 2 d following the addition of esculin compared with the control (days 4 and 5) to $7{\cdot}4 \times 10^9$ cfu/ml and thereafter declined slightly for the remainder of the experiment. The total number of coliforms in the test and control vessels was similar throughout (data not shown).

The initial SCFA concentrations in both fermenter vessels decreased slightly from 45 mM over the first 4 d during stabilisation. Following the addition of esculin, the total SCFA concentration in the test vessel increased to approximately 50 mM, a level greater than that in the

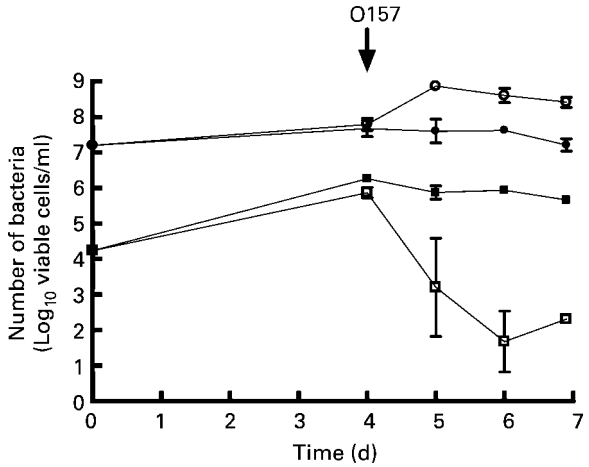

Fig. 4. Numbers of anaerobes and *Escherichia coli* O157 in rumen fermenters with and without the addition of esculin (↓; day 4). (•), Total anaerobes without esculin; (○), total anaerobes with esculin; (■), *E. coli* O157 strain 12900 without esculin; (□), *E. coli* O157 strain 12900 with esculin. Mean values are shown, with standard deviations represented by vertical bars.

control fermenter over the same period of time (data not shown). As expected, acetic acid was the major SCFA followed by propionic acid, then butyric acid.

Calf feeding trial

Before the experiment began it was demonstrated that calves accepted esculin when it was mixed throughout their solid feed at a concentration of 0·8 g esculin/kg feed. Throughout the experiment no deleterious effect on the health of the animals was observed. None of the animals were excreting *E. coli* O157 or rifampicin-resistant *E. coli*. In the 8 d following the first administration, *E. coli* O157 (12900^R) was detected in 37 % of faecal samples from the control group compared with 20 % of samples from the esculin-fed group. The faecal excretion of *E. coli* (total coliform count including all serogroups) was highest on days 2 and 3 (data not shown). *E. coli* O157 (12900^R) was excreted by all five animals in the control group on day 3 compared with two out of four of the esculin-fed group, although the number of *E. coli* O157 (12900^R) in positive samples was similar in both groups. One animal in the control group continued to excrete low levels of *E. coli* O157 (12900^R) up until day 9.

Discussion

In both the rumen and human colonic ecosystems the predominant bacteria belong to the low G + C Gram-positive phylum, including many members of clostridial groups, accompanied by *Bacteroides* and *Prevotella* species of the Cytophaga–Flexibacter–Bacteroides phylum of Gram-negative bacteria (Franks *et al.* 1998; Suau *et al.* 1999; Tajima *et al.* 1999; Ramsak *et al.* 2000; Pryde *et al.* 2002). The ability to hydrolyse esculin to the aglycone esculetin is a common feature of many of these organisms that possess β-glucosidase activities (Duncan, 2001).

The recognition of the role of farm animals as a potential reservoir of *E. coli* O157 (Rasmussen *et al.* 1993) has focused attention on how the survival of *E. coli* in the gastrointestinal tract might be influenced by the diet. *E. coli* is known to be sensitive to SCFA produced in fermentative gut compartments such as the rumen and colon (Wolin, 1969; Wallace *et al.* 1989). Diez-Gonzalez *et al.* (1998) hypothesised that the differences seen in the shedding of *E. coli* O157 by ruminants fed hay or grain might result from differences in the amounts of SCFA produced in the gut. It was argued that when grain is fed, *E. coli* strains in the rumen, including *E. coli* O157, become acid habituated, increasing survival during passage through the acid secretions of the ruminant stomach. Duncan *et al.* (1998) found that SCFA and plant coumarins showed additive effects in the inhibition of growth of *E. coli* O157. Thus, feeding diets rich in such plant secondary compounds might reduce the population size of *E. coli* O157 in the rumen and colon, where SCFA concentrations are high.

Naturally derived plant compounds provide a potentially simple and acceptable way to control the incidence and spread of *E. coli* O157 within farm animals. Whilst the potential for the expression of antimicrobial activity by plant secondary compounds in ruminant feeds is well documented (Van Soest, 1994), much of this work has concerned effects on the indigenous populations of anaerobes that inhabit the ruminant gastrointestinal tract. It is recognised that this microbial community is influenced by a range of plant secondary compounds, including tannins, saponins (Wallace *et al.* 1994), phenolic acids (Chesson *et al.* 1982), essential oils (Nagy & Tengerdy, 1968), alkaloids and coumarins (Moniello *et al.* 1996). The screening of plants or their compounds for antibacterial effects on

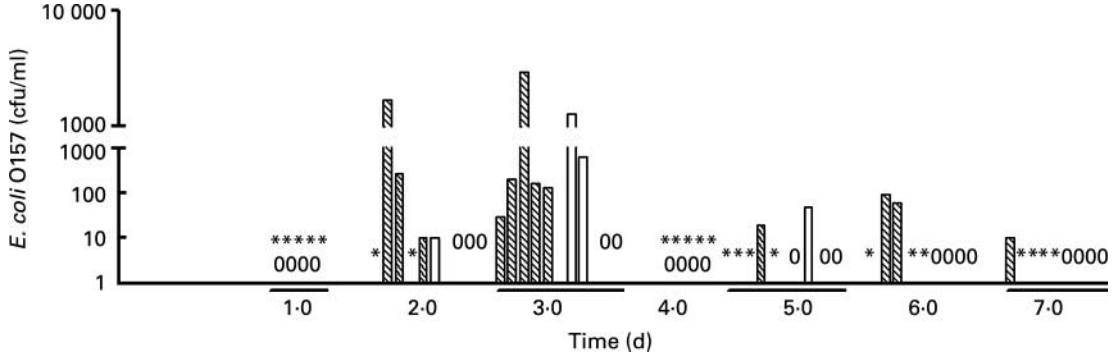

Fig. 5. Faecal numbers of marked *Escherichia coli* O157 shed by calves receiving (□; *n* 4) or not receiving (▨; *n* 5) esculin in the diet. The bars represent control calves 1 to 5 and esculin-fed calves 1 to 4 from left to right respectively. (*), Sample from the control calf group with no *E. coli* O157 detected; (0), sample from esculin-fed calf group with no *E. coli* O157 detected; cfu, colony-forming units.

E. coli O157 or other pathogens has been performed mainly using food plants or their constituents such as tea catechins (Toda *et al.* 1989) and essential oils (Hammer *et al.* 1999). However, surveys of the shedding of *E. coli* O157 by cattle in the USA have shown negative associations between shedding and the feeding of clover and of cottonseed, leguminous plants that contain a particularly rich variety of secondary plant compounds (Rasmussen *et al.* 1999). A dietary regimen including periods in which sheep were fed sagebrush grass, which contains essential oils with antibacterial properties, has been shown to reduce the shedding of *E. coli* O157 by sheep (Kudva *et al.* 1995).

The present study confirmed that esculin and esculetin maintained their inhibitory activity towards a strain of *E. coli* O157 when tested in both human colon- and rumen-simulating fermenter systems over a period of weeks. There was no indication of adaptation of the test strain, *E. coli* O157 (12900), during this time. These data suggest that a dietary supplement of esculin might reduce the numbers of *E. coli* O157 in the animal gut. They further suggest that the effect may be specific to the *E. coli* serogroup O157 while the overall coliform numbers were unaffected, which suggests that the mode of action has some strain- or serotype-based specificity. Genome sequencing shows that *E. coli* O157 differs in its gene complement to other *E. coli* (Ohnishi *et al.* 1999); it would not therefore be surprising if these genetic differences altered the susceptibility of the bacteria to specific inhibitors. The human colon fermenter medium used here was relatively rich in fermentable carbohydrate, whereas the rumen fermenter was supplied with mineral salts and received periodic 'feeds' of a pelleted sheep ration. In the rumen fermenter studies, the addition of esculin to the fermenter resulted in an increase in the total numbers of viable anaerobes. Esculin hydrolysis releases glucose, providing a growth substrate for anaerobes and resulting in enhanced SCFA levels which may have contributed to the decreased survival of *E. coli* O157.

In calves, the effect of esculin did not reach statistical significance. The results are nonetheless encouraging, as *E. coli* was detected less frequently in the esculin-fed group. The use of the marked rifampicin-resistant *E. coli* O157 strain (12900^{R}) and antibiotic selection agar in the animal studies allowed the detection of low levels of the organism in faecal samples.

Given the scarcity of the facilities for experiments with animals and the complex ethical issues involved, the *in vitro* model systems used in the present study can provide conditions similar to those in the gut, particularly in relation to turnover time and pH. They thus offer an alternative to batch incubations for the assessment of the possible effects of dietary additives on the survival of pathogens.

Recent observations on the distribution of *E. coli* O157 in cattle at slaughter suggest that the colon is an important habitat for this bacterium (Laven *et al.* 2003). For maximum protective effect, it may be necessary to ensure that compounds or treatments intended to inhibit the growth and survival of *E. coli* O157 reach the large bowel, perhaps by the use of timed-release encapsulation or other methods. More knowledge of the ecology of *E. coli* O157 in the digestive tract will help to improve nutritional strategies to reduce the incidence of this pathogen in farm animals.

Acknowledgements

We thank Kenneth Young for technical assistance and Greitje Zuur (BIOSS) for statistical advice, DEFRA for support of C. McW. L. and BBSRC for support of K. N. S. The Rowett Research Institute is funded by the Scottish Executive Environment and Rural Affairs Department (SEERAD).

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